EVALUATION OF THE METHANOGENIC STEP OF A TWO-STAGE ANAEROBIC DIGESTION PROCESS OF ACIDIFIED OLIVE MILL SOLID RESIDUE FROM A PREVIOUS HYDROLYTIC-ACIDOGENIC STEP

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Abstract

A study of the second step or methanogenic stage of a two-stage anaerobic digestion process treating two-phase olive oil mill solid residue (OMSR) was conducted at mesophilic temperature (35ºC). The substrate fed to the methanogenic step was the effluent from a hydrolytic-acidogenic reactor operating at an organic loading rate (OLR) of 12.9 g chemical oxygen demand (COD) L^{-1} d^{-1} and at a hydraulic retention time (HRT) of 12.4 days; these OLR and HRT were found to be the best values to achieve the maximum total volatile fatty acid concentration (14.5 g L^{-1} expressed as acetic acid) with a high concentration in acetic acid (57.5% of the total concentration) as the principal precursor of methane. The methanogenic stage was carried out in an anaerobic stirred tank reactor containing saponite as support media for the immobilization of microorganisms. OLRs of between 0.8 and 22.0 g COD L^{-1} d^{-1} were studied. These OLRs corresponded to HRTs of between 142.9 and 4.6 days. The methanogenic reactor operated with high stability for OLRs lower than 20.0 g COD L^{-1} d^{-1}. This behaviour was shown by the total volatile fatty acids/total alkalinity ratio, whose values were always kept ≤0.12 for HRTs > 4.6 days. The total COD (T-COD) removed was in the range of 94.3% to 61.3 % and the volatile solids (VS) removed between 92.8% and 56.1% for OLRs between 0.8 and 20.0 g COD L^{-1} d^{-1}. In the same way, a reduction of 43.8 % was achieved for phenolic content. The low concentration of total volatile fatty acids (TVFA) observed (below 1 g L^{-1} expressed as CH_3COOH) in the methanogenic reactor effluents showed the high percentage of consumption and conversion of these acids to methane. A methane yield of 0.268±0.003 L CH_4 at standard temperature and pressure conditions (STP) g^{-1} COD eliminated was achieved.

Keywords: Two-phase olive mill solid residue; two-stage anaerobic digestion process; methanogenic step.

1. Introduction

The changes produced in olive oil extraction systems with the implantation of the two-phase decanter or process generate a high humidity solid residue called two-phase olive mill solid residue (OMSR) as the principal and most pollutant waste (Borja et al., 2005). This waste is produced in a proportion of 800 kg ton^{-1} of olive processed. The two-phase decanter or two-phase process has been put into operation in a large number of mills in Spain, with 90% of the olive mills currently using this type of two-phase decanter as a consequence of the great reduction in the water consumption of the mills. The high generation of olive oil in Spain every year causes between 2 and 4 million tons of this waste annually (IOOC, 2004). The characteristics of this OMSR (low pH, high content in solids/organic matter, volatile fatty acids, presence of inhibitory compounds as poly-phenols, etc.) and the large quantities generated can pose large-scale environmental problems for Spain, taking the 2,000 Spanish olive oil factories into account, most of which are located in the Andalusian Community (Borja et al., 2005).

The anaerobic digestion process provides a good alternative for the treatment of medium/high organic load residues. The combined action of microorganisms working in two consecutive steps provides good waste stabilization, while generating a source of energy. The anaerobic digestion process is carried out by three principal groups of microorganisms: hydrolytics, acetogenics and methanogenics (Gujer and Zehnder, 1983). Nevertheless, the microorganisms which take part in the process of anaerobic digestion have different physiological requirements, nutrient requirements,

growing kinetics and levels of sensitivity to the environmental conditions. A separation of phases in the anaerobic digestion process provides good stability to the different groups of microorganisms and a more specific control of the conditions required for each one (Demirel and Yenigün, 2002). This separation in two stages allows the enrichment of the different populations of microorganisms (Cha and Noike, 1997) by means of the control of the operational parameters. Working in two stages or steps prevents the inhibition of the process because of the accumulation of inhibitory intermediate compounds. It is well known that the accumulation of intermediate metabolic products like volatile fatty acids can be a serious inconvenience of the methanogenic step (Veeken and Hamelers, 2000).

Another factor to take into consideration is that depending on the substrate treated the limiting step can be the hydrolytic-acidogenic step (Mata-Álvarez et al., 2000) or the methanogenic step (Labib et al., 1993). In this way, the physical separation of both phases can improve the performance to be achieved in each one.

Since the seventies, an extensive bibliography has detailed the benefits of two-stage anaerobic digestion, as working in stages means reaching higher efficiencies of the process, higher stability and better control when the appropriate conditions for every phase are provided. Although this kind of process is favourable for a large number of substrates, it has been shown that the two-stage process is not optimal for all kinds of wastes. A comprehensive study carried out with different kinds of substrates (Weiland, 1993) showed that the suitability of separation into two steps or the use of only one stage depends on the carbon/nitrogen (C/N) ratio. The C/N ratio influences the degradation capacity of a substrate by the microorganisms; this ratio also influences the stability of the process. In this way, substrates with C/N ratios of between 35 and 40 can be digested in one or two steps without significant differences

in the chemical oxygen demand removed. Nevertheless, for substrates with C/N ratios < 10, with high concentrations of proteins and nitrogen, the two-stage process was the best option.

The aim of this work was to study the methanogenic step of a two-stage anaerobic digestion process treating two-phase OMSR, providing an evaluation of the different operational parameters and the methane yield reached for the different OLRs and HRTs studied. With this in mind, the OMSR used was previously acidified in an acidogenic reactor. This initial step allowed the solubilisation of part of this substrate to achieve a high total volatile fatty acid (TVFA) concentration with a high percentage of acetic acid (Rincón et al., 2008a).

2. Materials and methods

2.1. Equipment

The first stage or hydrolytic-acidogenic stage was carried out in an anaerobic stirred tank reactor with an effective volume of 2.0 L; this equipment was described in detail elsewhere (Rincón et al., 2008a). The methanogenic stage was carried out in an anaerobic stirred tank reactor with an effective working volume of 1.8 L. To avoid loss of microorganisms with the reactor effluents, this reactor was provided with a 0.5 L settler situated at the top and with a low density (0.8 g mL^{-1}) magnesium silicate support media called saponite ($(Mg,Fe)_3(Si,Al)_4O_{10}(OH)_2.4H_2O$). The support media had a moisture content of 8.3% and a chemical composition of 4.4% Al_2O_3, 0.6% CaO, 2.0% Fe_2O_3, 1.0% K_2O, 25.4% MgO, 0.2% Na_2O, 57.3% SiO_2, 0.2% TiO_2, 8.3% calcinations loss, 220 m^2 g^{-1} specific surface area, 0.4-0.8 mm average size of particles and 49% porosity. The support material was commercially available (Minas de Gador S.A., Almería, Spain). Ensuring the immobilization of the biomass is very important since the

growth rate of the methanogen microorganisms is lower than the other microorganisms that take part in the anaerobic digestion process (Gujer and Zehnder, 1983).

This methanogenic reactor was provided with a magnetic stirrer, keeping an appropiate agitation level (260 rpm), and allowing an adequate transfer of inoculum and substrate. The reactor was fed manually on a daily basis by means of an external feeder. The liquid effluent was removed from the upper part of the settler through a hydraulic seal, comprising a 25 cm liquid column, which was designed to prevent air from entering the reactor and biogas from leaving it, and therefore maintaining the anaerobic environment during the process. The temperature was kept at the mesophilic range (35 ±2 ^{o}C).

The volume of CH_4 produced in the process was measured using an eight-litre Boyle-Mariotte reservoir fitted to the reactor (Field et al., 1988). CO_2 produced in the process was scrubbed by bubbling the gas mixture through a NaOH solution (3 M) before its entry into the reservoir. The remaining gas produced was collected by a water displacement system. The volume of water collected was equivalent to the volume of methane produced (Rincón et al., 2008a).

2.2. Inoculum

At the beginning of the experiments the reactor was inoculated with anaerobic sludge from an industrial anaerobic reactor located at a local brewery (Cruzcampo; Seville, Spain). The characteristics of the inoculum used were: pH, 8.1; total suspended solids, 34.9 g L^{-1}; mineral suspended solids, 8.9 g L^{-1}; volatile suspended solids, 26.0 g L^{-1}; total solids, 37.4 g L^{-1}; mineral solids, 11.0 g L^{-1}; and volatile solids, 26.4 g L^{-1}. These reported values were averages of triplicate samples with standard deviations lower than

5 % in all cases. The quantities used for the start-up of the reactor were: 1 L of sludge, 0.4 L of a nutrient-trace element solution and 0.4 L of distilled water to keep the effective reactor volume at 1.8 L.

The nutrient-trace element solution supplies the microorganisms implicated in the process with the nutrients necessary for bacteria growth, thus avoiding deficiencies during the experiments (Hickey et al., 1991). A detailed description of this solution is given elsewhere (Rincón et al., 2008a). The nutrients were only added at the beginning of the experiments, and no additional nutrients were added to the reactor after the start up.

The inoculum/support media ratio used was 1:1. This value was previously checked as a suitable ratio for keeping an adequate interaction inoculum/substrate (Borja et al., 1993a and 1993b). Higher concentrations of support can cause an increase in the apparent media density and viscosity, thus avoiding a proper mass transfer.

2.3. Feed characteristics

The substrate used in the experiments was two-phase olive mill solid residue (OMSR). This waste was collected from the experimental olive-oil factory located in the "Instituto de la Grasa" (CSIC), Seville (Spain). The olives processed were of the "Picual" variety with a low ripening level (2.5) (García and Yousfi, 2005) and were harvested at the beginning of the olive season (November 2003). The C:N:P (carbon, nitrogen, phosphorous) ratio of this waste was 28:1:0.2. The features and composition of this residue are shown in Table 1; total chemical oxygen demand (T-COD), soluble chemical oxygen demand (S-COD), particulate chemical oxygen demand (P-COD), soluble organic carbon (S-OC), total solids (TS), total mineral solids (MS), total volatile solids (VS), total suspended solids (TSS), mineral suspended solids (MSS), volatile

suspended solids (VSS), total volatile fatty acids (TVFA), partial alkalinity (Palk) and total alkalinity (Talk). The samples were stored at 4^{o}C to preserve the original characteristics of the residue.

Before the methanogenic experiments took place this OMSR was acidified in a hydrolytic–acidogenic reactor, working at an organic loading rate of 12.9 g COD L^{-1} d^{-1} (HRT=12.4 days) under controlled conditions until a solubilised substrate or acidified OMSR were obtained with a high concentration in volatile fatty acids and a high percentage in acetic acid as the principal precursor of methane (Rincón et al., 2008a).

The characteristics of the hydrolytic-acidogenic effluent or influent used for feeding the methanogenic reactor are summarized in Tables 1 and 2. As can be seen, the acidified OMSR had low pH and a total volatile fatty acid concentration of 14.5 g L^{-1} (expressed as CH_3COOH) with 57.5% acetic acid of the TVFA concentration.

2.4. Experimental procedure

Before starting the experiments, an adaptation or acclimatization of the inoculum to the substrate studied was carried out. Three different dilutions of acidified OMSR were used: 25%, 50% and 75%. The first dilution (25%) was used to keep the OLR between 0.5 and 1.5 g COD L^{-1} d^{-1}, the second dilution (50%) was used to increase the OLR between 1.5 g and 2.2 g COD L^{-1} d^{-1} and, finally, the third dilution (75%) was used to increase the OLR to 3 g COD L^{-1} d^{-1}. This acclimatization stage lasted around 45 days.

Once the biomass of the reactor was acclimated, the experiment was started using acidified OMSR (100%) and an organic loading rate of 0.8 g COD L^{-1} d^{-1}. During the experiments an ammonia solution (15%) was used to keep the substrate pH between 5.5 and 6.0 and thus improve the acetic acid consumption.

A total of 14 different experiments with OLRs from 0.8 g to 22.0 g COD L^{-1} d^{-1} corresponding to HRTs of between 142.9 and 4.6 days were carried out. The different OLRs and HRTs studied throughout the experiments are shown in Table 3. This table also shows the different flow-rates fed to the reactor in order to reach the increasing OLRs. Each experiment corresponding to the different OLRs lasted at least twice the corresponding hydraulic retention time to ensure the steady-state conditions desired. Once the steady-state conditions were achieved for each run studied (when the deviations between the observed values of the consecutive measurements of a specific parameter were less than 5%) the samples were collected for analysis over a period of at least five consecutive days. The pH and CH_4 volume produced were determined daily. T-COD, S-COD, P-COD, S-OC, TS, MS, VS, TSS, MSS, VSS, TVFA, Palk, Talk and total phenolic compounds were determined in the effluents obtained at the steady-state for each OLR and HRT studied.

2.5. Chemical Analyses

The analyses performed during the experimental runs were carried out according to the recommendations of the Standard Methods of APHA (APHA, 1989). Specifically, T-COD, S-COD and P-COD were determined according to method number 5220 C, while TS, MS, VS, TSS, MSS and VSS were analysed according to method numbers 2540B and 2540E. Palk and Talk were determined using method 2320B. pH was analyzed with a pH-meter (Crison, model basic 20). S-OC was measured using a Dohrmann DC-190 analyser after filtrating the samples with a 0.45 μm acetate filter (Whatman). Gas chromatographic analyses were carried out for determination of the total volatile fatty acids and partial volatile fatty acid species (acetic, propionic, butyric, isobutyric, valeric and isovaleric acids). A detailed description of the gas

chromatograph used is given elsewhere (Rincón et al., 2008a). The TVFA/Talk ratio (expressed in Equivalents of acetic acid/Equivalents of $CaCO_3$) can be used as a measurement of system stability in anaerobic processes. Ratios remained below the threshold of 0.4 show optimum performances without risk of acidification (Fannin, 1987).

Phenolic compounds were extracted beforehand (Romero et al., 2002) and measured at 725 nm by spectrophotometry using the Folin-Ciocalteau method (Rincón et al., 2007).

The pH and the CH_4 volumes produced were determined daily. Once the steady state was achieved, samples were collected for the analytical determinations over 5 consecutive days, constituting 5 different samples to ensure that representative data were obtained.

In the OMSR, carbon and nitrogen were determined using a LECO Microanalyzer CHNS-932 (Leco Corporation, St Joseph, MI, USA), infrared and thermal conductivity detectors were used. Phosphorous was measured by spectrophotometry at 880 nm, using the normalized methods 4500-P, B and E. Finally, oils and fats were analysed by Soxhlet extraction with n-hexane using the official method of the EEC N^{o}2568/91 (European Community Official Diary, L248/1 of 05.09.1991).

3. Results and discussion

3.1. Evolution of the pH and alkalinity (partial and total) with the OLR

Figure 1 shows the evolution of the pH and partial and total alkalinity with the OLR (g COD L^{-1} d^{-1}). As can be seen, the pH was very stable for organic loading rates in the range of 0.8 to 20.0 g COD L^{-1} d^{-1}, fluctuating around 7.0, the highest value observed was 8.2.

In addition, sufficient alkalinity levels were observed in the reactor, that aided in buffering the pH levels during the experiment. The values of total alkalinity increased with the OLRs studied from 5.37 g L^{-1} (expressed as $CaCO_3$) at an OLR of 0.8 g COD L^{-1} d^{-1} to 7.21 g L^{-1} at an OLR of 6.5 g COD L^{-1} d^{-1}. They were constant for OLRs of between 6.5 and 10.5 g COD L^{-1} d^{-1}. For higher OLRs the alkalinity decreased slightly from 6.55 to 5.75 g L^{-1} at the end of the experiments, with 5.75 g L^{-1} corresponding to an OLR of 22.0 g COD L^{-1} d^{-1}. The partial alkalinity (or bicarbonate alkalinity) behaved in a similar way. However, for an OLR of 17.0 g COD L^{-1} d^{-1}, the partial alkalinity decreased more noticeably, as a consequence of the consumption of bicarbonates due to the increase in volatile fatty acids in the system as will be shown in section 3.5.

Experimental data obtained from a previous study (Borja et al., 2002) indicated that a total alkalinity of 1700 mg L^{-1} as $CaCO_3$ was sufficient to prevent the pH from dropping below 7.0 in the anaerobic digestion of diluted OMSR (80%) at OLR of 12 g COD L^{-1} d^{-1}. In addition, pH values of 6.9 or higher were found for OLRs lower than 12 g COD L^{-1} d^{-1} and HRTs higher than 12.5 days when OMSR 80% was processed, with pH 7.2 being the maximum value achieved.

3.2. Evolution of solids and total, soluble and particulate chemical oxygen demand (T-COD, S-COD, P-COD) with the OLR

As the experiments progressed and the HRTs made shorter, the concentration of organic matter in the effluents taken from the methanogenic reactor was higher. In this way T-COD, S-COD and P-COD concentrations increased with decreased HRT. The T-COD increased from 5.6 to 42.8 g L^{-1}, the S-COD from 2.8 to 20.0 g L^{-1} and the P-COD increased from 2.8 g L^{-1} to 22.8 g L^{-1} when the HRT decreased from 142.9 to 4.6 days (OLR increased from 0.8 to 22.0 g COD L^{-1} d^{-1}). As can be seen, the values obtained for

the P-COD were very similar to the S-COD, although sometimes the P-COD concentrations were higher than the S-COD concentrations. For OLRs higher than 8.6 g COD L^{-1} d^{-1} (HRT<12.3 days) the P-COD was always higher than the S-COD, as can be seen in Figure 2.

The influents fed to the methanogenic reactor came from a hydrolytic-acidogenic reactor. In this reactor, a large amount of the easily degradable matter of the OMSR was previously eliminated and transformed into volatile fatty acids (acidified OMSR), before going into the methanogenic reactor. A part of the P-COD was also eliminated at the first stage, which reduced the concentration of P-COD from 104.5 g L^{-1} in the OMSR to 54.5 g L^{-1} in the effluent of the hydrolytic-acidogenic reactor. Nevertheless, P-COD degradation is more complicated and slower than the degradation of S-COD because the insoluble fraction, or P-COD, must go through a previous stage of solubilisation before its conversion into soluble compounds, volatile fatty acids and finally into methane. In this way, the P-COD contribution to the T-COD concentration was very similar to the S-COD contribution at the longer HRTs and lower OLRs, but for OLRs>8.6 g COD L^{-1} d^{-1} and HRTs<12.3 days the P-COD was always higher than the S-COD at the effluents of the methanogenic step.

Figure 2 also illustrates the evolution of the total volatile fatty acid (TVFA) concentration (in units of g COD L^{-1}) with the OLR. As can be seen, the concentration of TVFA at the effluents of the reactor was very low throughout the process and for all the OLRs and HRTs studied. It suggested that most of the volatile fatty acids were consumed in this methanogenic step and transformed into methane without accumulation in the system. As can be seen in Figure 2, the contribution of the TVFA in units of chemical oxygen demand to the T-COD is very low in all cases.

3.3. Evolution of the solids with the hydraulic retention time

The effect of the HRT on the total and suspended solids is shown in Figure 3 (a) and (b) respectively. The values of total solids (TS) and volatile solids (VS) increased in the effluents of the methanogenic reactor from 10.5 to 46.7 g L^{-1} and from 4.8 to 35.6 g L^{-1} respectively, when the HRT decreased from 142.9 to 4.6 days. Solid effluent concentrations increased progressively with a decrease in the HRTs applied in the methanogenic step of the two-stage anaerobic process.

Figure 4 shows the variation of the volatile solids removed (VS removed), total and soluble chemical oxygen demand removed (T-COD removed and S-COD removed) with the OLR. The VS removed decreased from 92.8% to 56.1% when OLR increased from 0.8 to 20.0 g COD L^{-1} d^{-1}, and HRT decreased from 142.9 to 5.0 days, respectively. For OLRs > 20.0 g COD L^{-1} d^{-1} (HRTs < 5.0 days) the VS removed was 46.1%. T-SCOD and S-COD removed decreased from 94.3% to 61.3% and from 93.8% to 68.9% respectively for the same intervals of HRTs and OLRs. For HRT < 5.0 days the values of T-COD removed and S-COD removed decreased to 56.9% and 55.6%, respectively. All values of organic matter removal decreased when the OLR increased and HRT decreased. For HRTs < 10.5 days and OLRs > 10.5 g COD L^{-1} d^{-1}, the VS removed were lower than both T-COD and S-COD removed. This can be explained because the content in volatile solids at the effluents takes into account both the soluble compounds, the very easily biodegradable ones, and the insoluble compounds (or compounds in suspension) that must be hydrolyzed and transformed into soluble compounds and volatile fatty acids in order to be eliminated. For the same reason the T-COD removed (which joins both T-COD and P-COD) was lower than the S-COD removed.

3.4. Biodegradability

The influent fed to the methanogenic reactor or acidified OMSR had a higher content in soluble compounds than the original OMSR. However, not all the influent fed to the methanogenic reactor was soluble and biodegradable, as was shown in section 3.2. Figure 5 shows the variation of T-COD removed with the HRT in the methanogenic step. As can be seen, the T-COD removed kept values of between 94% and 93% for the longest HRTs studied (142.9 and 52.9 days), but these percentages of T-COD removed decreased to values lower than 93% for HRTs that were shorter than 30.0 days. Therefore, it can be suggested that 94% of the organic matter content of the acidified OMSR was biodegradable in the methanogenic reactor and only 6% of the organic matter was non-biodegradable, independently of the HRT studied. The non-biodegradable matter would be composed of the liquid and solid non-biodegradable fraction, the rest of microorganisms and the cellular membranes that are not easily biodegradable.

Lower T-COD removal efficiencies (82.9%) were obtained in the one-stage anaerobic digestion of diluted OMSR (80%) at OLRs>12 g COD L^{-1} d^{-1} (Borja et al., 2002).

3.5. Evolution of the total volatile fatty acid (TVFA) concentration and composition

Table 2 summarises the total and individual acid concentrations of the acidified OMSR fed to the methanogenic step. Most of the volatile fatty acids fed to the methanogenic reactor were removed with a high conversion of these intermediate products into methane. This elimination was clear taking into account the low TVFA concentrations in the methanogenic effluents, whose values were less than 1 g L^{-1} (expressed as acetic acid) for all the OLRs studied, including very high OLRs such as

20.0 g COD L^{-1} d^{-1} (Figure 6). The TVFA concentration was only increased over 1 g L^{-1} for the last OLR studied - 22.0 g COD L^{-1} d^{-1} corresponding to a HRT of 4.6 days. For this OLR, a TVFA concentration of 3 g L^{-1} was achieved in the effluents of the methanogenic step. This sudden increase showed that the TVFA concentration fed from the hydrolytic-acidogenic reactor was not consumed in this case. This increase in TVFA brought about a decrease in the pH, alkalinity (Figure 1) and methane production. Specifically, the pH decreased from 7.5 to 6.9 when the OLR increased from 20.0 to 22.0 g COD L^{-1} d^{-1}.

The analysis performed by gas chromatography at the effluents of the methanogenic reactor showed that the predominant volatile fatty acids were acetic and propionic acids, with concentrations higher than other acids (butyric acid, iso-butyric acid, valeric acid and iso-valeric acid). The concentrations of acetic acid ranged between 32.7% and 70.9%, while the propionic acid concentrations varied between 16.3% and 34.8%. The variation of the different volatile fatty acid concentrations with the OLR in the methanogenic reactor is shown in Figure 6. It can be appreciated in this figure that the acetic acid concentration was between 219 mg L and 389 mg L^{-1} for all the OLRs, and it only increased to 900 mg L^{-1} for the last OLR studied (22.0 g COD L^{-1} d^{-1}). This concentration was higher than the inhibitory acetic acid concentrations reported in the bibliography, where it was shown that concentrations higher than 788 mg L^{-1} caused failure in the process and low stability (Hill et al., 1987). The propionic and valeric acid concentrations reported in previous works for a correct working process in anaerobic reactors were below 741 mg L^{-1} and 1021 mg L^{-1}, respectively (Ahring et al., 1995). In the present methanogenic reactor a maximum concentration of propionic acid of 555 mg L^{-1} was achieved at the highest OLR studied, and this concentration was always below the failure limit value mentioned in the literature. However, for the valeric acid,

although its concentration was very low for all the OLRs and HRTs studied, its value increased up to 1905 mg L^{-1} at an OLR of 22.0 g COD L^{-1} d^{-1}. Therefore, it has been proved that this valeric acid concentration was completely inhibitory for the system and, more specifically, for the methanogens which were completely inhibited.

3.6. Evolution of volumetric methane production rates (rCH_4) and the TVFA/Talk ratio

The TVFA/Talk ratio remained below the threshold of 0.4 for optimum performance (Fannin, 1987) and the ratio was below 0.12 for OLRs as high as 20.0 g COD L^{-1} d^{-1} (Figure 7). The variations of the TVFA/Talk ratio and the volumetric methane production rates (L CH_4 L^{-1} d^{-1}) with OLR are showed in Figure 7. This ratio increased above 0.4 for OLR > 22.0 g COD L^{-1} d^{-1} (HRT = 4.6 days), indicating the upper limit for OLR around 20 g COD L^{-1} d^{-1} for stable operation.

In addition, as was previously reported (Borja et al., 2002), the TVFA/Talk ratio values were also lower than the failure limit value in the anaerobic digestion of diluted two-phase OMSR (80%) for OLRs lower than 9 g COD L^{-1} d^{-1} (HRTs> 16.6 days). However, when the HRT decreased to 10 days, a considerable increase of the TVFA/Talk ratio up to a value of 0.95 was observed, which brought about a clear destabilization of the process.

At the beginning of the experiments, the volumetric methane production rate increased proportionally with the OLR, from 0.160 L CH_4 L^{-1} d^{-1} for an OLR = 0.8 g COD L^{-1} d^{-1} (HRT=142.9 days) to 2.96 L CH_4 L^{-1} d^{-1} for an OLR=15.5 g COD L^{-1} d^{-1} (HRT of 6.4 days). When the OLR increased up to 20.0 g COD L^{-1} d^{-1} (HRT=5.0 days) the values of the volumetric methane production rate increased slightly to 3.24 L CH_4 L^{-1} d^{-1}, decreasing finally after this OLR to 2.603 L CH_4 L^{-1} d^{-1} at an OLR of 22.0 g COD L^{-1} d^{-1}. The maximum methane production rate (3.24 L CH_4 L^{-1} d^{-1}) achieved at the

methanogenic step of the two-stage anaerobic digestion process of OMSR was 90.5% higher than that observed in the one stage anaerobic digestion process of this substrate (1.7 L CH_4 L^{-1} d^{-1}) (Rincon et al., 2008b). In addition, it was reached at an OLR of 20 g COD L^{-1} d^{-1}, a much higher value than that necessary to achieve the maximum methane production rate at the one-stage process.

In the same way, the maximum methane production rate achieved in the present study was 53% higher than that obtained in the one-stage anaerobic digestion of diluted OMSR (80%) at mesophilic temperature (Borja et al., 2002), maximum value achieved at an OLR of 12 g COD L^{-1} d^{-1}, equivalent to a HRT of 12.5 days.

The methanogenic step of the two-stage anaerobic digestion process of this substrate was very stable for a long range of OLRs and HRTs and at an OLR as high as 20.0 g COD L^{-1} d^{-1}. These high OLRs were also achieved in methanogenic reactors of two-stage anaerobic digestion processes of other substrates such as cattle slurry with OLR values of up to 15.0 g COD L^{-1} d^{-1} (Demirer and Chen, 2005), food waste with OLRs of up to 15.8 g COD L^{-1} d^{-1} (Shin et al., 2001) and some energetic crops with OLRs of up to 25 g COD L^{-1} d^{-1} (Andersson and Björnsson, 2002). The behaviour observed in the present step can be attributed to the fact that the influent fed to the methanogenic reactor was an effluent from a hydrolytic-acidogenic reactor, in which the OMSR was pre-digested and solubilized, with the production of very high concentrations of TVFA and especially of acetic acid. These characteristics favoured the direct action of the methanogenic microorganisms, the acetic acid being the immediate precursor of the methane. In general, the OLRs reached in anaerobic digesters working in only one stage are not so high, only in EGSB or UASB reactors. These OLR values could be achieved when operating with very easily biodegradable substrates (Franklin, 2001).

3.7. Specific methane yield

The methane yield coefficient, Y_p, was calculated using equation [1], assuming that the volume of gas produced per day, q_{CH4}, is proportional to the amount of substrate consumed (Rincón et al., 2008b).

$$q_{CH4} = Y_p\, q[(\text{T-COD})_o\text{-}(\text{T-COD})_{st}] \qquad [1]$$

In this equation: $(\text{T-COD})_o$ is the initial total chemical oxygen demand at the digester inlet (in g L^{-1}) and $(\text{T-COD})_{st}$ is the total chemical oxygen demand at the digester effluents at steady-state for every OLR and HRT studied; q_{CH4} is the volume of methane obtained for every OLR and HRT studied (L CH_4 d^{-1}); q is the flow-rate of substrate fed to the reactor (L d^{-1}); and, finally, Y_p is the methane yield coefficient (in L CH_4 g^{-1} COD eliminated). The values of methane were corrected at standard temperature and pressure (STP) conditions.

Plotting equation [1] in the form q_{CH4} against $q[(\text{T-COD})_o\text{-}(\text{T-COD})_{st}]$ (Figure 8), a value of 0.268±0.003 L CH_4 STP g^{-1} COD eliminated was obtained for the methane yield coefficient with 95% of confidence limits and a determination coefficient R^2=0.9986. This methane yield coefficient value was 10% higher than that observed in the one-stage anaerobic digestion process of this substrate (0.244 L CH_4 STP g^{-1} COD removed) (Rincón et al., 2008b). This methane yield was also considerably higher than that obtained in the one-stage anaerobic digestion of diluted two-phase OMSR (80%) at mesophilic temperature (0.20 L CH_4 STP g^{-1} COD removed) (Borja et al., 2002).

The maximum production of methane achieved in the present reactor was 5.834 L CH_4 d^{-1}, and this production was reached at an OLR as high as 20 g COD L^{-1} d^{-1}. Similar OLRs were achieved in the methanogenic steps of two-stage anaerobic digestion processes of different substrates (Shin et al., 2001; Andersson and Björnsson, 2002; Demirer and Chen, 2005; Parawira et al., 2005).

3.8. Phenolic compounds eliminated

One of the characteristics that makes the OMSR highly pollutant is its concentration in poly-phenolic compounds. These kinds of compounds, with very complex structures, are highly inhibitory for the anaerobic digestion process and especially for the methanogenic microorganisms (Fedorack and Hrudey, 1984; Borja et al., 1997). The concentration in phenolic compounds into the OMSR was reduced in a 40.7% of its initial value in the first stage (hydrolytic-acidogenic) being the initial concentration into the influent fed to the methanogenic reactor 8.89 g L^{-1} (expressed as caffeic acid). This previous elimination of phenolic compounds at the first stage could help to improve the performance of the methanogenic step, being the concentration of these compounds in the final effluents 5 g L^{-1} (20.0 g COD L^{-1} d^{-1}) and reaching a removal of 43.8%.

4. Conclusions

The results of this study demonstrate that the methanogenic degradation of acidified olive mill solid residue from a previous hydrolytic-acidogenic reactor is very effective. High organic matter removals were achieved with values of T-COD eliminations of between 94.3% and 61.3% and VS removals of between 92.8% and 56.1% for OLRs in the range of 0.8 to 22.0 g COD L^{-1} d^{-1} (HRTs in the range of 142.9-4.6 days). The two-stage anaerobic digestion process showed a high stability in every step: hydrolytic-acidogenic and methanogenic. In the methanogenic step, this high stability was maintained over a wide OLR range (OLRs from 0.8 to 20.0 g COD L^{-1} d^{-1}) and until very short HRTs (5 days). Low concentrations of TVFA (less than 1 g L^{-1} expressed as acetic acid) were kept during all the OLRs studied, which showed that the high concentrations of acids from the hydrolytic-acidogenic stage and fed into the

methanogenic reactor were easily removed for OLRs ≤ 20.0 g COD L^{-1} d^{-1} and HRT ≥ 5 days. It can be concluded that the methanogenic step of the previously acidified olive mill solid residue from a two-phase olive oil manufacturing system, helped achieve very stabilised effluents as well as a high methane yield (0.268±0.003 L CH_4 STP g^{-1} COD eliminated) with a considerable reduction in all pollutant parameters.

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Table 1

Characteristics of the two-phase olive oil mill residue (OMSR)* and the acidified OMSR* used as substrate in the methanogenic stage

Parameters	OMSR*	Acidified OMSR*
pH	5.3	6.0
Palk (as $CaCO_3$)	-	0.4
Talk (as $CaCO_3$)	1.1	7.7
TVFA (as acetic acid)	1.4	14.5
T-COD	162.0	99.4
S-COD	57.5	45.0
P-COD	104.5	54.4
S-OC	22.20	12.5
TS	143.0	----
MS	17.0	----
VS	126.0	66.0
TSS	106.0	----
MSS	11.0	----
VSS	95.0	----
Phosphorous	0.0035	----
Oils and fats	2.2 %	----
Moisture	86.7 %	----
Total phenols (as caffeic acid)	15.0	8.89

* All units are expressed in g L^{-1} except the moisture, oils, fats and pH. Values are averages of six determinations; there was virtually no variation (less than 5 %) between analyses.

Table 2

Total volatile fatty acid concentration (TVFA) in g L^{-1} and individual acid concentrations in % (C2: acetic acid, C3: propionic acid, i-C4: iso- butyric acid, C4: butyric acid, i-C5: iso-valeric acid and C5: valeric acid) of the acidified OMSR used as feed of the methanogenic step

TVFA ($g\ L^{-1}$ as acetic acid)	C2 (%)	C3 (%)	i-C4 (%)	C4 (%)	i-C5 (%)	C5 (%)
14.5	57.5	10.9	15.2	14.2	3.6	0.3

Table 3.

Flow-rates (q), organic loading rates (OLR) and hydraulic retention times (HRT) studied at the methanogenic step.

OLR (g COD L^{-1} d^{-1})	q (L d^{-1})	HRT (days)
0.8	0.013	142.9
2.0	0.034	52.9
3.5	0.060	30.0
5.0	0.086	21.0
6.5	0.111	16.2
8.6	0.146	12.3
10.5	0.172	10.5
12.8	0.230	7.8
14.0	0.253	7.1
15.5	0.280	6.4
17.0	0.310	5.8
18.5	0.345	5.2
20.0	0.362	5.0
22.0	0.395	4.6

Figure captions

Figure 1. Evolution of the pH, Talk and Palk with the OLR.

Figure 2. Variation of the T-COD, S-COD, P-COD and the TVFA (expressed in units of chemical oxygen demand) with the OLR.

Figure 3. Variation of the total, mineral and volatile solids (TS, MS and VS) (a) and total, mineral and volatile suspended solids (TSS, MSS and VSS) (b) with the different HRTs studied

Figure 4. Variation of the T-COD, S-COD and VS removed with the OLR.

Figure 5. Variation of the total chemical oxygen demand (T-COD) removed with the HRT.

Figure 6. Evolution of the total and individual volatile fatty acids with the OLR.

Figure 7. Effect of the OLR on the volumetric methane production rate and on the total volatile fatty acids/total alkalinity ratio in the effluents.

Figure 8. Variation of the volume of methane produced per day (q_{CH4}) as a function of the product of the differences of substrate concentrations at the reactor inlet ($T\text{-}COD_o$) and outlet ($T\text{-}COD_{st}$) and the feed flow-rate (q).

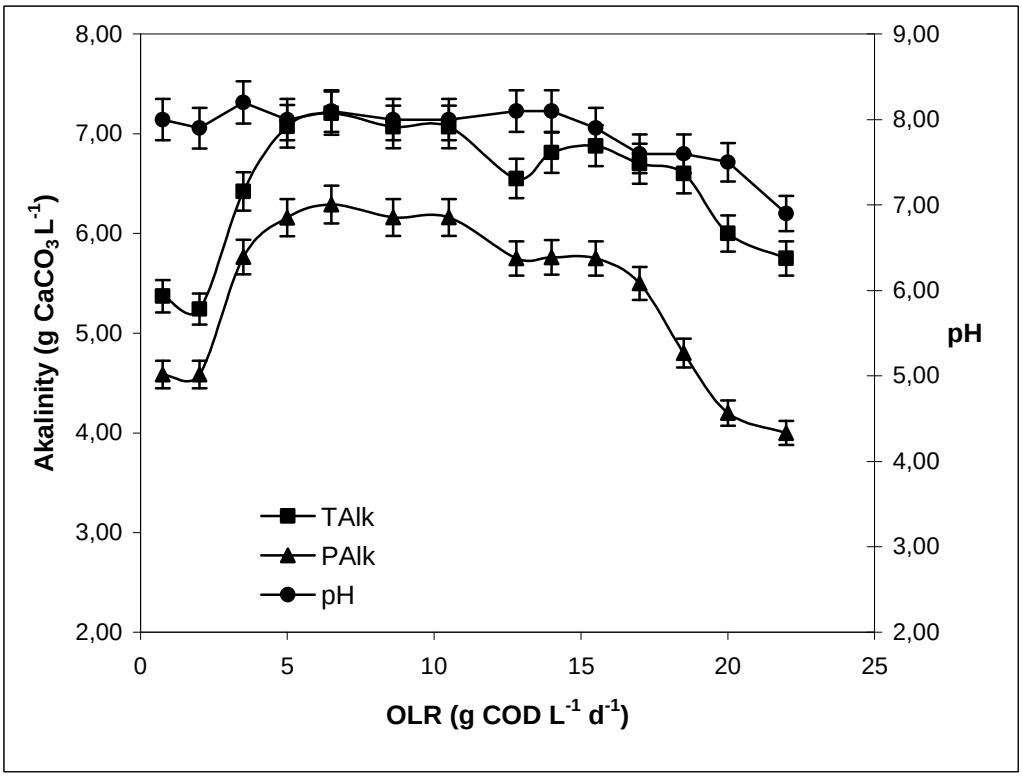
8,00
7,00
6,00
5,00
4,00
3,00
2,00
9,00
8,00
7,00
6,00
5,00
4,00
3,00
2,00
0
5
10
15
20
25
Akalinity (g CaCO3 L-1)
pH
OLR (g COD L-1 d-1)
TAlk
PAlk
pH

Figure 1

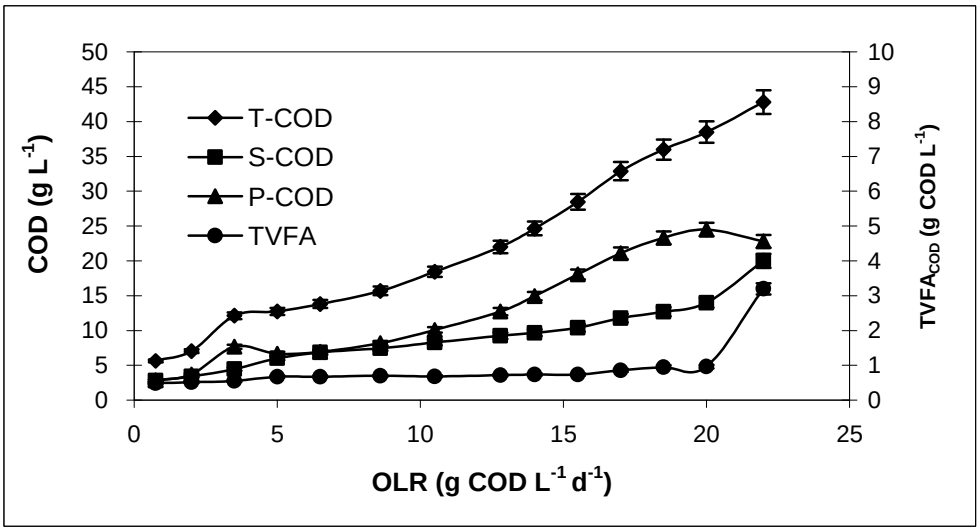
T-COD
S-COD
P-COD
TVFA
COD (g L-1)
TVFACOD (g COD L-1)
OLR (g COD L-1 d-1)
0
5
10
15
20
25
30
35
40
45
50
1
2
3
4
6
7
8
9

Figure 2

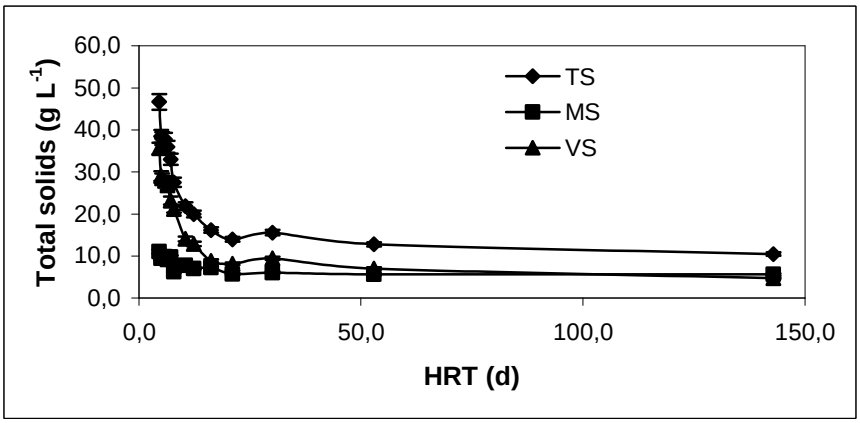

Figure 3 (a)

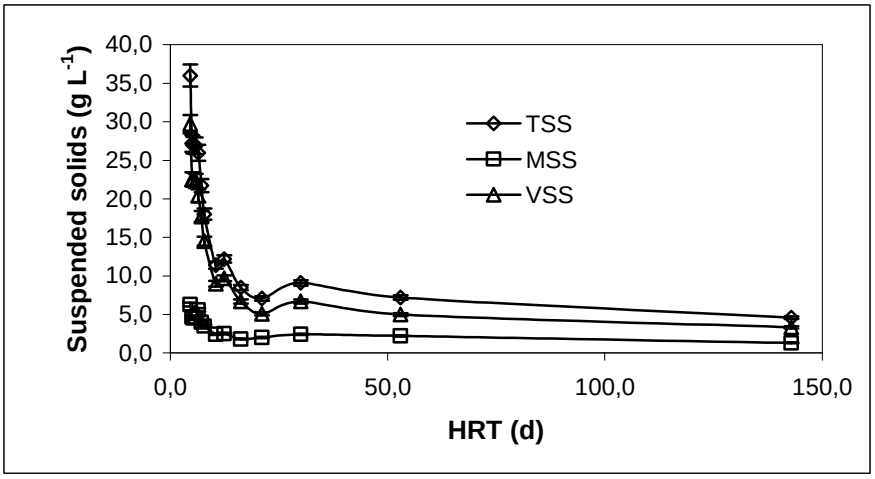

Figure 3 (b)

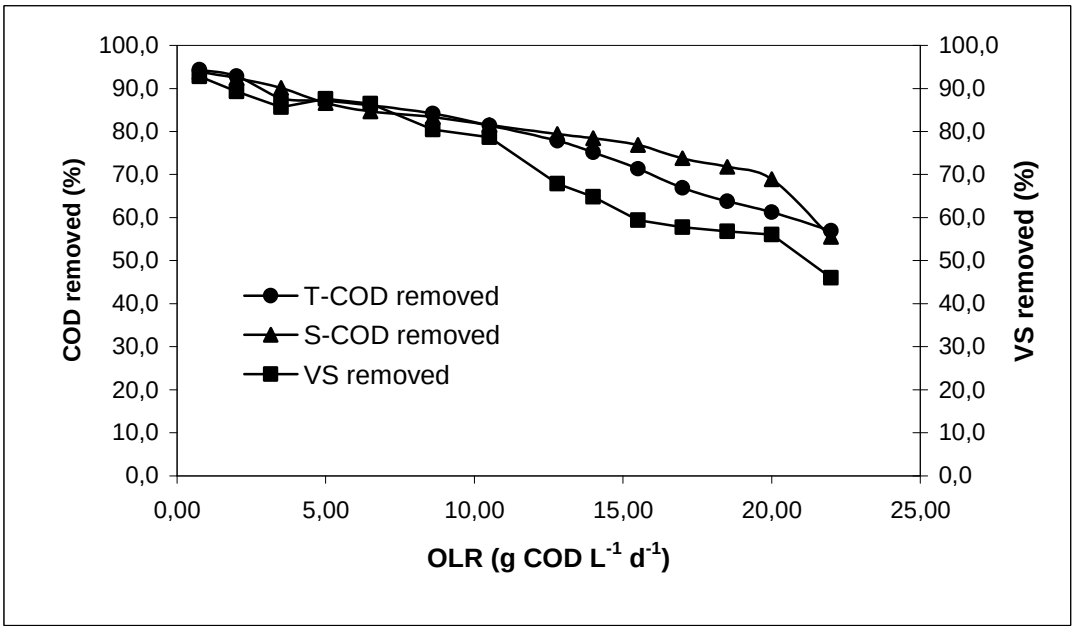
100,0
90,0
80,0
70,0
60,0
50,0
40,0
30,0
20,0
10,0
0,0
COD removed (%)
VS removed (%)
T-COD removed
S-COD removed
VS removed
0,00
5,00
10,00
15,00
20,00
25,00
OLR (g COD L-1 d-1)

Figure 4

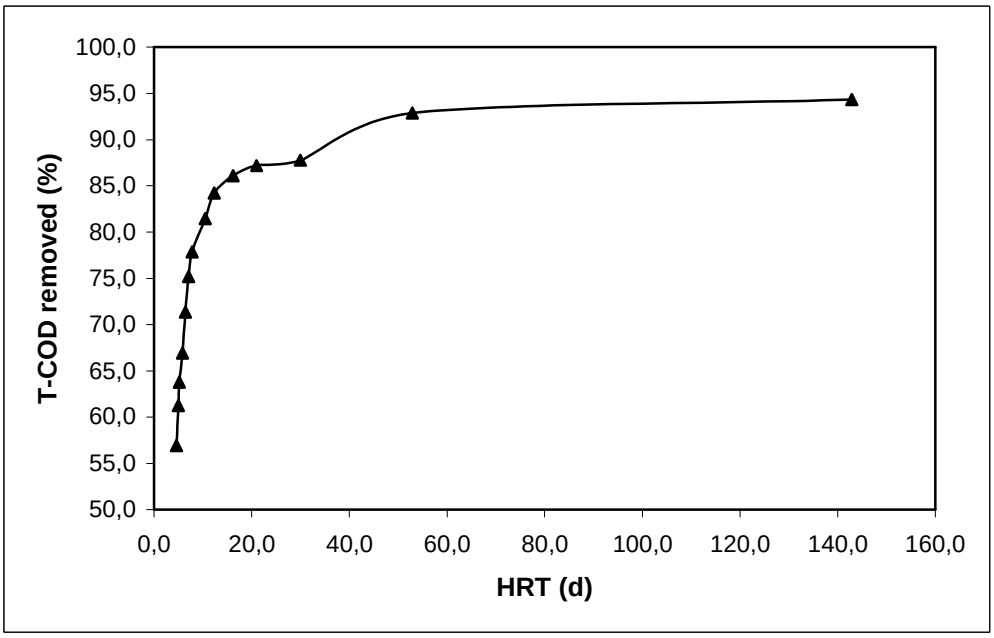
100,0
95,0
90,0
85,0
80,0
75,0
70,0
65,0
60,0
55,0
50,0
T-COD removed (%)
0,0
20,0
40,0
60,0
80,0
100,0
120,0
140,0
160,0
HRT (d)

Figure 5

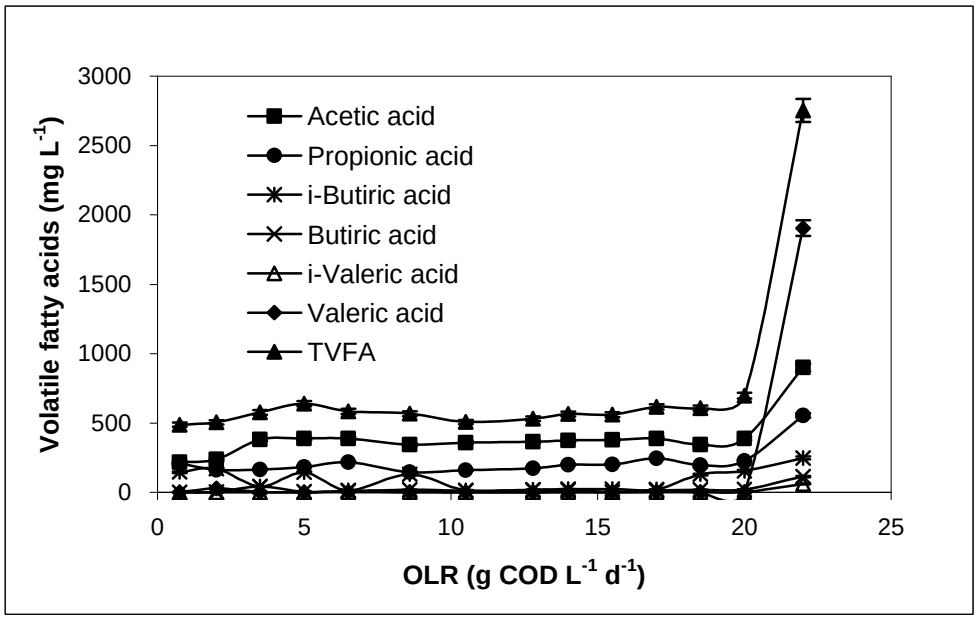
Volatile fatty acids (mg L-1)
3000
2500
2000
1500
1000
500
0
0
5
10
15
20
25
OLR (g COD L-1 d-1)
Acetic acid
Propionic acid
i-Butiric acid
Butiric acid
i-Valeric acid
Valeric acid
TVFA

Figure 6

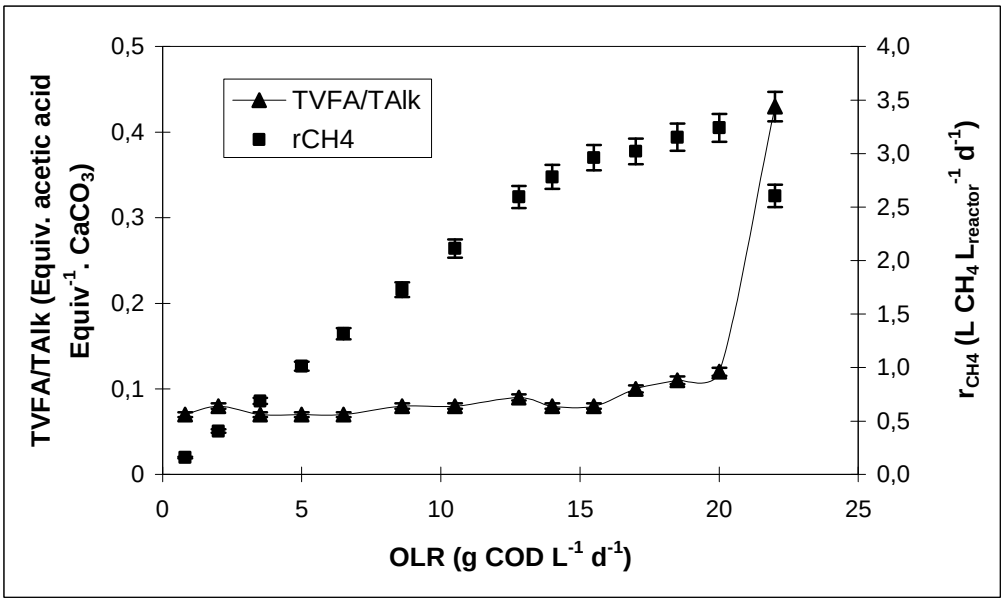
TVFA/TAlk
rCH4
TVFA/TAlk (Equiv. acetic acid Equiv-1. CaCO3)
rCH4 (L CH4 Lreactor-1 d-1)
OLR (g COD L-1 d-1)
0
0,1
0,2
0,3
0,4
0,5
0,0
0,5
1,0
1,5
2,0
2,5
3,0
3,5
4,0
0
5
10
15
20
25

Figure 7

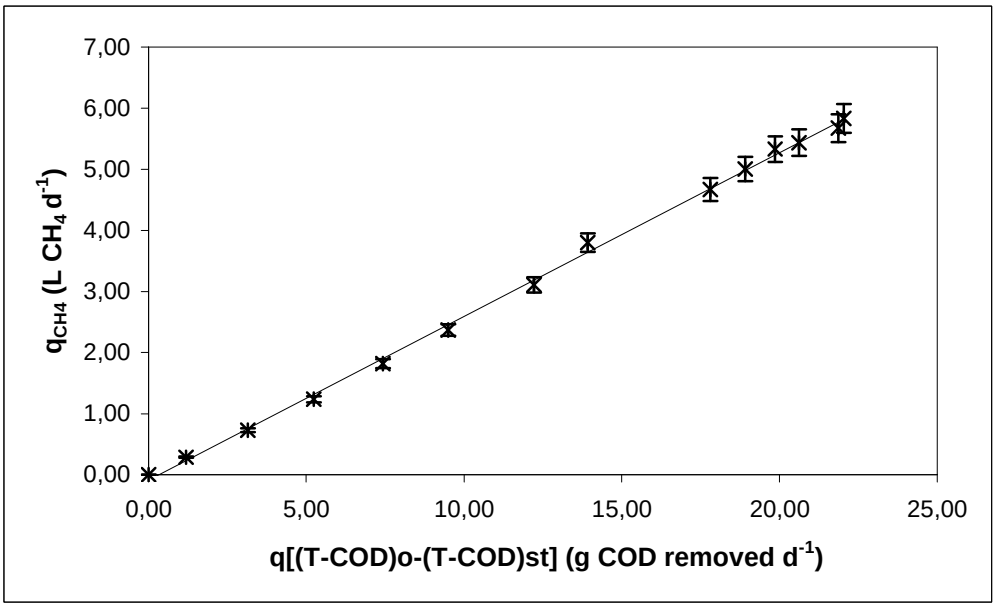
7,00
6,00
5,00
4,00
3,00
2,00
1,00
0,00
q_{CH4} (L CH_4 d^{-1})
0,00
5,00
10,00
15,00
20,00
25,00
q[(T-COD)o-(T-COD)st] (g COD removed d^{-1})

Figure 8